DEPARTMENT OF HEALTH AND HUMAN SERVICES

MEMORANDUM OF CONFERENCE
September 20, 1994

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Subject: BXN Cotton

Introduction

This meeting was intended to bring to closure Calgene's consultation regarding BXN Cotton. BXN cotton was genetically engineered to contain a nitrilase which confers resistance to the herbicide bromoxynil. Calgene first consulted regarding the modified cotton in August of 1992 (SBJ 1319). Calgene submitted, on June 14, 1994, a summary of its safety assessment. Subsequently, on September 19, 1994, Calgene submitted additional information in support of conclusions they stated in the June 14, 1994 submission.

Introduced Genetic Material

Calgene noted that the binary vectors used to transform cotton were analogous to those used in the generation of FLAVR SAVR tomatoes and therefore, the safety of most sequences within the T-DNA borders of the binary plasmids, including the *kan*^{r} gene, have previously been addressed (21 CFR 173.170 and 21 CFR 573.130; Summary of Consultation with Calgene, Inc., concerning FLAVR SAVR tomatoes, May 17, 1994). Calgene described the identity and function of the additional sequences inserted into the T-DNA and introduced into BXN cotton including the BXN gene and associated regulatory sequences (summarized on pp. 19-21 of the September 19 submission).

The BXN gene (encoding a bromoxynil-degrading nitrilase) was isolated from a *Klebsiella* species obtained from a soil sample. The nucleotide sequence of the 1.2 kb DNA segment encoding the nitrilase was determined. Calgene stated that for expression in plants, all but 11 bp of the 5' and 96 bp of the 3' untranslated region of the gene were used. Calgene also stated that it has generated physical maps of the inserts present in all nine of the transgenic cotton lines they have developed from independent transformants. In all cases, only one or two copies of the *kan*^{r} gene and the BXN gene were introduced into the transgenic cotton genomes. Based on genetic and molecular analysis, Calgene has concluded that the transgene sequences are integrated at one or two sites.

Identity and Function of Expression Products Encoded by the Introduced Genetic Material

Two new proteins, namely the enzymes aminoglycoside 3'-phosphotransferase II (APH(3')II, a product of the *kan*^{r} marker gene) and a nitrilase (the product of the BXN gene) are expressed in BXN cotton. APH(3')II was used in the selection of successfully transformed cotton plants. The nitrilase enzyme, which is composed of two identical subunits (each is a 37 kd protein), confers the bromoxynil-resistant phenotype on BXN cotton because it catalyzes the conversion of bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) to 3,5-dibromo-4-hydroxybenzoic acid, rendering bromoxynil ineffective as a herbicide. Calgene described experiments using related substrates, which showed that the nitrilase encoded by the BXN gene is highly specific for bromoxynil.

Safety of the Introduced Proteins

Calgene noted that the safety of APH(3')II in the development of new varieties of cotton has already been addressed (21 CFR 173.170 and 21 CFR 573.130) and therefore, did not address it further other than to note that it is expressed at very low levels.

With respect to establishing the safety of the BXN gene-encoded nitrilase, Calgene noted that the major cotton product consumed by humans in the U.S. is refined cottonseed oil and that nitrilase has been confirmed not to be present in oil derived from BXN cottonseed, as expected (refined oils generally do not contain any protein). Calgene stated that nitrilase is found at very low amounts in unprocessed whole cottonseed, cottonseed hulls, and cottonseed meal which are used in animal feed and therefore, exposure of animals to nitrilase would be low. Calgene has determined that the enzyme makes up about 0.0006% of seed protein which corresponds to 0.00006 % of meal protein.

Calgene stated that the biological function of the introduced nitrilase protein does not raise any safety concern and the protein is not reported to be toxic nor have significant homology with known toxins. Calgene stated that nitrilases are ubiquitous throughout the plant and microbial kingdoms and have been described in many important food and animal feed crops, suggesting that intake of nitrilases from food sources and exposure to nitrilases from microorganisms has been taking place over a long period of time. Calgene noted that in plants, nitrilases are postulated to be important in the manufacture of the plant growth hormone indole acetic acid.

As mentioned above, Calgene first noted that the nitrilase protein is not expected to be present in refined oil and therefore, will not be in human food. Nonetheless, Calgene assessed the allergenic potential of the introduced nitrilase to demonstrate that there would be no risk even if human exposure were to occur. First, Calgene stated that the introduced nitrilase does not have characteristics common to allergens such as glycosylation, protease-stability, and presence in food at high concentrations (summarized on pp. 13-15 of the September 19 submission). Secondly, Calgene stated that a sequence homology search in the Entrez database (which is provided by the National Center for Biotechnology Information at NIH and contains all major DNA and protein databases from the USA, Europe and Japan) showed that the introduced nitrilase protein has no significant sequence homology to known allergens.

Compositional Analysis

Endogenous Toxicants

Calgene presented data on the levels of gossypol and cyclopropenoid fatty acids in whole cottonseed from BXN cotton and from standard cotton varieties grown in three different locations. Calgene stated that the data support the conclusion that gossypol and cyclopropenoid fatty acid levels in the transgenic cotton lines fall within the levels seen in common varieties of cotton and are not significantly different from those found in the parental variety (data summarized on pp. 1-5 of September 19 submission).

Concentration and Bioavailability of Important Nutrients

Calgene discussed the results of their analysis of the fatty acid composition of cottonseed oil derived from BXN cotton lines and from the parental line. The data showed that the fatty acid composition of cottonseed oil from BXN cotton lines is comparable to that obtained for the parental variety and falls within the ranges provided in the Codex Standard 22-1981 (Codex Alimentarius) for edible cottonseed oil. Because cottonseed and cottonseed meal are used in animal feed, Calgene also analyzed for the following in BXN cotton lines and in the parental variety: % total protein in meal, % residual oil in meal, amino acid composition of meal, and crude fiber, acid detergent fiber, and neutral detergent fiber in whole seeds. Calgene stated that in all of these components, the values obtained for BXN cotton were well within the normal range for cotton.

Conclusions

Calgene has concluded, in essence, that the BXN cotton they have developed is not significantly altered within the meaning of 21 CFR 170.30(f)(2) when compared to cotton varieties with a history of safe use. At this time, based on Calgene's description of its data and analysis, the agency considers Calgene's consultation on this product to be complete.

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